

CLAIMS

1. A method for preparing a ligand presenting assembly (LPA) enabling presentation of desired sequence(s) comprising the steps of

- (a) providing by solid phase synthesis or fragment coupling ligands comprising desired sequence(s), the ligands being attached to a solid phase,
- (b) if necessary, deprotecting any N-terminal amino groups while the ligand(s) are still attached to the solid phase,
- (c) reacting the ligand(s) having unprotected N-terminal amino groups with an achiral di-, tri- or tetracarboxylic acid so as to provide a construct having a ring structure, and
- (d) cleaving the construct from the solid phase so as to provide an LPA comprising ligands having free C-terminal groups.

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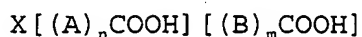
2. A method according to claim 1 for the preparation of an LPA enabling presentation of desired sequence(s) or desired sequence(s) and chemical moieties further comprising the steps of

- (c¹) if present, prior to step (d), deprotecting any N-protected amino groups originating from the carboxylic acid used in step (c),
- (c²) continuing the solid phase synthesis or fragment coupling so as to provide ligand(s) comprising desired sequence(s) having at least one N-protected N-terminal amino group and/or attaching chemical moieties, and

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(c³) if present, deprotecting any N-terminal amino group(s) prior to or after step (d).

3. The method according to claim 1 or 2, wherein the achiral acid
5 used in step (c) is of the general formula



wherein n and m independently are an integer of from 1 to 20, X is
10 HN, A and B independently are a substituted or unsubstituted C₁₋₁₀
alkyl, a substituted or unsubstituted C₂₋₁₀ alkenyl, a substituted
or unsubstituted cyclic moiety, a substituted or unsubstituted
heterocyclic moiety, or a substituted or unsubstituted aromatic
moiety, or A and B together form a substituted or unsubstituted
15 cyclic moiety, a substituted or unsubstituted heterocyclic moiety,
or a substituted or unsubstituted aromatic moiety, or

n and m are 0 or an integer of from 1 to 20, X is H₂N(CR₂)_pCR, or
RHN(CR₂)_pCR, wherein p is 0 or an integer of from 1 to 20, wherein
20 each R is H, a substituted or unsubstituted C₁₋₁₀ alkyl, a
substituted or unsubstituted C₂₋₁₀ alkenyl, a substituted or
unsubstituted cyclic moiety, a substituted or unsubstituted
heterocyclic moiety, or a substituted or unsubstituted aromatic
moiety, and A and B are both a substituted or unsubstituted C₁₋₁₀
25 alkyl, a substituted or unsubstituted C₂₋₁₀ alkenyl, a substituted
or unsubstituted cyclic moiety, a substituted or unsubstituted
heterocyclic moiety, or A and B together form a substituted or
unsubstituted cyclic moiety, a substituted or unsubstituted
heterocyclic moiety, or a substituted or unsubstituted aromatic
30 moiety, or

n and m are 0 or an integer of from 1 to 20, X is HO(CR₂)_pCR,
HS(CR₂)_pCR, halogen-(CR₂)_pCR, HOOC(CR₂)_pCR, ROOC(CR₂)_pCR, HCO(CR₂)_pCR,

RCO(CR₂)_pCR, or [HOOC(A)_n][HOOC(B)_m]CR(CR₂)_pCR, wherein p is 0 or an integer of from 1 to 20, each R independently is H, a substituted or unsubstituted C₁₋₁₀ alkyl, a substituted or unsubstituted C₂₋₁₀ alkenyl, a substituted or unsubstituted cyclic moiety, a substituted or unsubstituted heterocyclic moiety, or a substituted or unsubstituted aromatic moiety, and A and B are both a substituted or unsubstituted C₁₋₁₀ alkyl, a substituted or unsubstituted C₂₋₁₀ alkenyl, a substituted or unsubstituted cyclic moiety, a substituted or unsubstituted heterocyclic moiety, or A and B together form a substituted or unsubstituted cyclic moiety, a substituted or unsubstituted heterocyclic moiety, or a substituted or unsubstituted aromatic moiety, or

n and m are 0 or an integer of from 1 to 20, X is H₂N(CR₂)_p, RHN(CR₂)_p, HO(CR₂)_p, HS(CR₂)_p, halogen-(CR₂)_p, HOOC(CR₂)_p, ROOC(CR₂)_p, HCO(CR₂)_p, RCO(CR₂)_p or [HOOC(A)_n][HOOC(B)_m], wherein p is 0 or an integer of from 1 to 20, each R independently is H, a substituted or unsubstituted C₁₋₁₀ alkyl, a substituted or unsubstituted C₂₋₁₀ alkenyl, a substituted or unsubstituted cyclic moiety, a substituted or unsubstituted heterocyclic moiety, or a substituted or unsubstituted aromatic moiety, and A and B together form a substituted or unsubstituted cyclic moiety, a substituted or unsubstituted heterocyclic moiety, or a substituted or unsubstituted aromatic moiety.

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4. The method according to any one of claims 1-3, wherein the achiral acid is a di- or tricarboxylic acid.

5. The method according to any one of claims 1-4, wherein the achiral acid is selected among imino diacetic acid, 2-amino malonic acid, 3-amino glutaric acid, 3-methylamino glutaric acid, 3-chloro glutaric acid, 3-carboxymethyl glutaric acid, 3-methoxy-carbonyl glutaric acid, 3-acetyl glutaric acid, glutaric acid,

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tricarballic acid, 3,4-bis-carboxymethyl adipic acid, 4-(2-carboxyethyl)-pimelic acid, (3,5-bis-carboxymethylphenyl)-acetic acid, 3,4-bis-carboxymethyl-adipic acid, benzene-1,2,4,5-tetra carboxylic acid, 4-(3-carboxy-allylamino)-but-2-enoic acid, 4,4'-
 5 imino-dibenzoic acid, 1,4-dihydropyridine-3,5-dicarboxylic acid, 5-amino isophthalic acid, 2-chloro malonic acid, 3-hydroxy glutaric acid, and benzene-1,3,5-tricarboxylic acid.

6. The method according to any one of claims 1-5, wherein the
 10 desired sequence is a peptide comprising naturally occurring amino acids and/or non-naturally occurring amino acids, a peptide nucleic acid (PNA) sequence or a peptidomimetic.

7. The method according to any one of claims 2-6, wherein the
 15 chemical moiety is an entity enhancing the solubility or immunogenicity of the LPA obtained according to claims 1-5, or is an entity suitable for directing the LPA to its target, or a marker.

8. The method according to claim 7, wherein the chemical moiety is
 20 selected from fatty acids, antibodies or peptides for directing the LPA to its target, fluorophores, biotin, enzymes such as horse radish peroxidase, alkaline phosphatase and soya bean peroxidase, or nucleic acid sequences.

9. The method according to any one of claims 1-8, wherein the
 25 desired sequence comprises all or part of one or more B cell epitopes, all or part of one or more T cell epitopes, or all or part of one or more B and T cell epitopes, or mimics thereof.

10. The method according to claim 9, wherein at least one of the
 30 sequences is derived from a sequence, wherein the C-terminal amino acids are important for an immune response.

11. The method according to any one of claims 1-10, wherein the desired sequence is derived from viruses, bacteria, toxins, allergens, autoimmune system-related compounds, cancer related compounds, cell adhesion molecules, neurotropic factors, fungi or parasites, or a sequence homologous thereto.

12. The method according to any one of claims 1-11, wherein the desired sequence is derived from OspC protein of *Borrelia burgdorferi* or is a homologous sequence capable of reacting with anti-OspC antibodies or of provoking an immune response.

13. The method according to claim 12, wherein the LPA obtained provide a C-terminal presentation of the C-terminal sequence Pro-Lys-Lys-Pro of OspC.

14. The method according to any one of claims 1-11, wherein the desired sequence is derived from the flagellum of *Borrelia burgdorferi* or is a homologous sequence capable of reacting with anti-flagellum antibodies.

15. The method according to claim 12 or 13, wherein the LPA obtained provide C-terminal presentation of sequence(s) derived from OspC of *Borrelia burgdorferi* and further comprises desired sequence(s) derived from the flagellum of *Borrelia burgdorferi*.

16. The method according to any one of claims 1-11, wherein the desired sequence is derived from *Mycobacterium tuberculosis*.

17. The method according to claim 12 and 13, wherein the LPA obtained further comprises desired sequence(s) derived from *Mycobacterium tuberculosis*.

18. The method according to claim 16 or 17, wherein the desired

sequence comprises the ESAT-6, 51-70 sequence protein or the ESAT-6, 1-17 sequence protein of Mycobacterium tuberculosis.

19. An LPA obtainable by the method defined in claims 1-18.

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20. An LPA according to claim 19, wherein the ligands comprising the desired sequence is selected from a peptide comprising naturally occurring and/or non-naturally occurring amino acids, a PNA sequence or a peptidomimetic.

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21. An LPA according to claim 19 or 20, wherein the chemical moiety is an entity enhancing the solubility or immunogenicity of the LPA of claims 1-20 or is an entity suitable for directing the LPA to its target, or a marker.

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22. An LPA according to any one of claims 19-21, wherein the chemical moiety is selected from fatty acids, antibodies or peptides for directing the LPA to its target, fluorophores, biotin, enzymes such as horse radish peroxidase, alkaline phosphatase and soya bean peroxidase, or nucleic acid sequences.

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23. An LPA according to any one of claims 19-22, wherein the desired sequence comprises all or part of one or more B cell epitopes, all or part of one or more T cell epitopes, or all or part of one or more B and T cell epitopes, or mimics thereof.

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24. An LPA according to claim 23, wherein at least one of the sequences is derived from a sequence, wherein the C-terminal amino acids are important for an immune response.

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25. An LPA according to any one of claims 19-24, wherein the desired sequence is derived from viruses, bacteria, toxins, allergens, auto-immune system-related compounds, cancer related

compounds, fungi or parasites, or a sequence homologous thereto.

26. An LPA according to any one of claims 19-24, wherein the desired sequence is derived from the OspC protein of *Borrelia burgdorferi*, or is a homologous sequence capable of reacting with anti-OspC antibodies or of provoking an immune response.

27. An LPA according to claim 26, wherein the LPA obtained provide a C-terminal presentation of the C-terminal sequence Pro-Lys-Lys-Pro of OspC.

28. An LPA according to any one of claims 19-27 selected from

[LPA-I]: FmocN(CH₂CO-ProValValAlaGluSerProLysLysPro-OH)₂,

[LPA-II]: biotin-NH(CH₂)₅CON(CH₂CO-ProValValAlaGluSerProLysLysPro-OH)₂,

[LPA-III]: NH₂CH(CH₂CO-ProValValAlaGluSerProLysLysPro-OH)₂, and

[LPA-IV]: H-Lys-NHCH(CH₂CO-ProValValAlaGluSerProLysLysPro-OH)₂.

29. An LPA according to any one of claims 19-25, wherein the desired sequence is derived from the flagellum of *Borrelia burgdorferi* or is a homologous sequence capable of reacting with anti-flagellum antibodies.

30. An LPA according to any one of claims 26-28 further comprising desired sequence(s) derived from the flagellum of *Borrelia burgdorferi* or is a homologous sequence capable of reacting with anti-flagellum antibodies.

31. An LPA according to any one of claims 19-25, wherein the

desired sequence is derived from *Mycobacterium tuberculosis*.

32. An LPA according to any one of claims 26-28 further comprising desired sequence(s) derived from *Mycobacterium tuberculosis*.

33. An LPA according to claim 31 or 32, wherein the desired sequence comprises the ESAT-6, 51-70 sequence protein or the ESAT-6, 1-17 sequence protein of *Mycobacterium tuberculosis*.

34. An LPA according to any one of claims 31-33 selected from

[LPA-V]: ^{Seq ID 1} (HO-ProLysLysProSerGluAlaValValPro-COCH₂)₂CH-NH-Lys-
^{Seq ID 2} (GlnLeuAlaAsnAsnLeuGluThrAlaThrAlaAspTrpLysGlnGlnValGlyGlnTyr-H)₂,
and

[LPA-VI]: ^{Seq ID 1} (HO-ProLysLysProSerGluAlaValValPro-COCH₂)₂N-Lys (AlaSer-
^{Seq ID 3} AlaAlaAlaGluIleGlyAlaPheAsnTrpGlnGlnGluThrMet-H)₂.

35. An immunological composition for raising an immune response in an animal, including a human being, comprising an LPA as defined in any of claim 1-34.

36. An immunological composition according to claim 35 comprising an LPA as defined in any of claims 1-34 in combination with an adjuvant.

37. An immunological composition according to claim 35 or 36 which is in the form of a vaccine.

38. A vaccine according to claim 37 for oral or parenteral, e.g. subcutaneous, intramuscular, intradermal, nasal, or pulmonary administration.

39. A method for generating antibodies in an animal, including a human being, which method comprises administering to said mammal an antibody-generating amount of the LPA as defined in any of claims 1-34.

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40. A kit for use in the diagnosis of infections caused by viruses, bacteria, toxins, allergens, autoimmune system-related compounds, cancer related compounds, cell adhesion molecules, neurotropic factors, fungi or parasites, which kit comprises an LPA as defined
10 in any of claims 1-34 together with means for detecting or visualising binding between the LPA and the substance to be detected.

41. A kit for use in diagnosing diseases caused by *Borrelia burgdorferi sensu lato*, which kit comprises an LPA as defined in
15 any of claims 26-30 or 32-34 together with means for detecting or visualising binding between the LPA and the substance to be detected.

42. Use of an LPA according to any one of claims 19-34 for
20 preparing a pharmaceutical composition for the treatment, alleviation or prophylaxis of diseases caused by viruses, bacteria, toxins, allergens, autoimmune system-related compounds, cancer related compounds, cell adhesion molecules, neurotropic factors,
25 fungi or parasites.